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ANATOMY AND PRELIMINARY CHEMICAL ANALYSIS OF THE POST-PHARYNGEAL GLANDS OF THE LEAF-CUTTING ANT, *ACROMYRMEX OCTOSPINOSUS* (REICH.) (HYM., FORMICIDAE)

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SUMMARY

The post-pharyngeal glands of *Acromyrmex octospinosus* are a pair of glove-shaped glands lying over the brain and opening into the pharynx. A single layer of cells, lined on the inside with cuticle, make up the wall of the gland. There is a complex system of muscles where the gland joins the gut, which probably control the release of the gland contents into the gut.

The lumen of the gland contains a yellow oil which is mostly a mixture of about 10 triglycerides. Minor components include free fatty acids and sterols.

The gland contents may serve as a supplementary diet for the larvae.

RÉSUMÉ

L'anatomie et l'analyse chimique préliminaire des glandes pharyngiennes de la fourmi manioc, *Acromyrmex octospinosus* (Reich.) (Hym., Formicidae)

Les glandes pharyngiennes d'*Acromyrmex octospinosus* sont des glandes paires et en forme de gants, situées au-dessus du cerveau et débouchant dans le pharynx. Une couche monocellulaire bordée d'une cuticule à l'intérieur en constitue les parois. A l'endroit où la glande rencontre l'œsophage, il y a un système complexe de muscles qui contrôlent probablement la libération du contenu glandulaire dans l'œsophage.

La lumière de la glande contient une solution jaune qui est essentiellement constituée d'un mélange d'environ 10 triglycérides. Les constituants mineurs comprennent des acides gras et des stérols libres.

Il se peut que le contenu de la glande serve d'aliment supplémentaire pour les larves.

INTRODUCTION

Leaf-cutting ants of the genera *Acromyrmex* and *Atta* are serious agricultural pests in the neotropics (Amante, 1964; Nickel, 1958; Wheeler, 1907; Wille, 1929). *Acromyrmex octospinosus* (Reich) is widely distributed through the Panamanian region, the West Indies and the northern countries on the South American mainland (Gonçalves, 1961).

Our interest in the post-pharyngeal glands of leaf-cutting ants results from an observation by Echols (1966) on *Atta texana* (Buckley). While studying the acceptability of poison baits to *A. texana*, Echols noticed

that the post-pharyngeal glands became strikingly coloured when the ants fed on dyed soya bean oil. Soya bean oil is used to dissolve the insecticide in some leaf-cutting ant baits (CHERRETT, 1969) and is claimed to be the ingredient that attracts *A. texana* (ECHOLS, 1966). Recently PEREGRINE *et al.* (1972) showed that insecticides dissolved in the soya bean oil should behave in a similar way to the dye, so that the gland may play a part in spreading toxicant around the colony as ECHOLS suggested (1966). This problem might be resolved if we understood how the gland functions.

The aim of this work was to obtain anatomical and chemical information about the gland that might explain its significance in the biology of the insect.

MATERIALS AND METHODS

I. — Anatomy and histology.

Winged queens of *A. octospinosus*, obtained from laboratory colonies originally from Trinidad, were used for all studies. The colonies were reared at 27° ± 2°C; 80-90 % relative humidity with 12 hours light and 12 hours dark, conditions in which the winged sexual forms are readily produced.

The internal anatomy of the head was studied on fresh specimens dissected under water. To enhance the contrast between different tissues, crystals of methylene blue were dropped on to the dissections, left for a few minutes and the excess stain washed off. The cuticular endoskeleton of the head was examined after boiling the specimens gently in 10 % potassium hydroxide solution.

For the histological investigation, dissected glands were dropped into Carnoy's fixative (with chloroform) or 10 % neutral buffered formalin, dehydrated in graded alcohols, cleared in chloroform, and embedded in « Fibrowax » (Arnold Horwell Ltd.). Sections were cut at 5 µm and stained with Ehrlich's haematoxylin and eosin.

II. — Pharyngeal gland contents.

Both the oil released from the gland on dissection and hexane-diethyl ether (1 : 1) extracts of whole dissected glands were analysed.

a) THIN LAYER CHROMATOGRAPHY (TLC)

The lipids of the post-pharyngeal gland were separated into different classes on thin layer plates of Silica Gel F254 (20 × 20 cm, 0.25 mm thick) prewashed with methanol. The plates were developed with a petroleum ether (B.P. 60-80° C)-diethyl ether-acetic acid (70 : 30 : 1) mixture. Relative amounts of gland components were estimated quantitatively by comparison with synthetic lipid mixtures of known composition. Detection was with iodine vapour and also by charring with chromic acid-concentrated sulphuric acid solution.

Intact lipids were isolated by scraping bands from the TLC plate and eluting with a hexane-diethyl ether (1 : 1) mixture or acetone.

b) MASS SPECTROMETRY

Pharyngeal gland oil, and lipids of the gland isolated by TLC, were fractionated in the direct inlet system of a Hitachi-Perkin Elmer RMU6E double focussing mass spectrometer. The mass spectrometer was operated at a chamber temperature of 250° C with an ionising voltage of 70 eV and the direct inlet sample heater varied between 50° and 420° C.

OBSERVATIONS

I. — Morphology of the anterior alimentary canal.

The terminology used in this account is that employed by JANET (1905) in his anatomical description of the head of *Lasius niger* Reine.

The *intima* of the foregut of *Acromyrmex octospinosus* is particularly well developed in the buccal and pharyngeal regions and supports a complex system of buccal and pharyngeal muscles.

The *mouth* (fig. 1 and 2, *m*) is slit-like and leads into a very short *buccal tube* (buc. tu.). The cuticle making up the *buccal tube* and *anterior pharyngeal region* (ant. phar.) is fan-shaped. When the muscles of this region are relaxed, the two plates of cuticle making up the floor and roof are closely applied to one another. The *posterior pharynx* (post. phar.) is a narrow region more sclerotised than the anterior region. Several muscles (see below) are associated with this region. The paired *post-pharyngeal glands* (post. phar. gls.) open into the *posterior pharynx* (post. phar.). From here the alimentary canal narrows, forming the *oesophagus* (oes.), which connects with the crop in the gaster.

II. — Musculature of the buccal and pharyngeal regions.

The muscles associated with the anterior part of the fore gut can be conveniently separated into ventral (inferior) and dorsal (superior) muscles.

There are two ventral muscles (fig. 2). Anteriorly, a large *inferior dilatory muscle* (inf. dil. mus.) is inserted on the pharynx. Posteriorly, a paired *inferior retractor muscle* (inf. ret. mus.) is associated with the oesophagus. Both these muscles originate

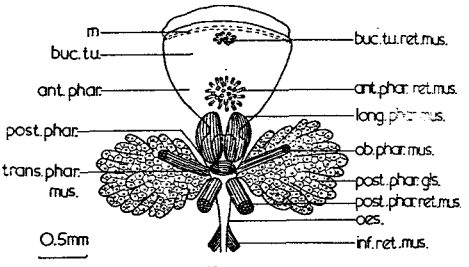

FIG. 1.

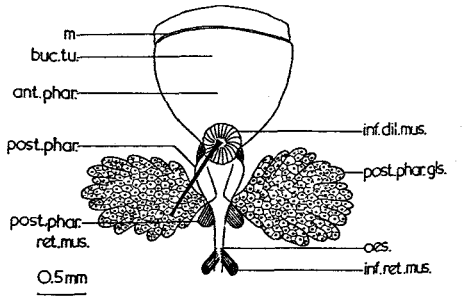

FIG. 2.

FIG. 1. — Bucco-pharyngeal region of *Acromyrmex octospinosus*-dorsal aspect. *ant. phar.*, anterior pharynx; *ant. phar. ret. mus.*, anterior pharyngeal retractor muscle; *buc. tu.*, buccal tube; *buc. tu. ret. mus.*, buccal tube retractor muscle; *inf. ret. mus.*, inferior retractor muscle; *long. phar. mus.*, longitudinal pharyngeal muscle; *m*, mouth; *ob. phar. mus.*, oblique pharyngeal muscle; *oes.*, oesophagus; *post. phar.*, posterior pharynx; *post. phar. gls.*, post-pharyngeal glands; *post. phar. ret. mus.*, post-pharyngeal retractor muscle; *trans. phar. mus.*, transverse pharyngeal muscle.

FIG. 2. — Bucco-pharyngeal region of *Acromyrmex*- ventral aspect. *inf. dil. mus.*, inferior dilatory muscle; other abbreviations as for fig. 1.

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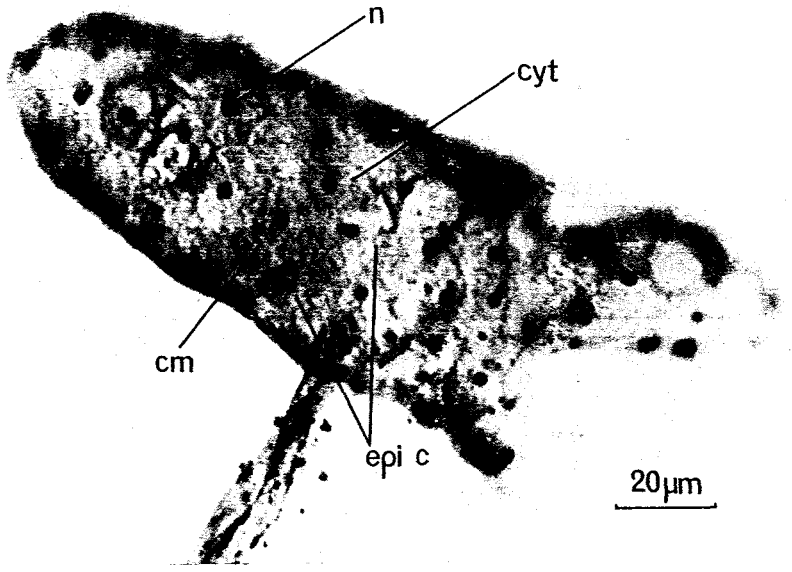

FIG. 3. — Whole mount of part of the post-pharyngeal gland, vitally stained with methylene blue.
cm., cell membrane; *cyt.*, cytoplasm; *epi. c.*, epithelial cells; *n*, cell nucleus.

on the transverse bar of cuticle which forms the floor of the occipital foramen at the back of the head.

There are six dorsal muscles, three of which are paired (fig. 1). The *buccal tube retractor* muscle (buc. tub. ret. mus.) is a small median muscle which originates on the inner surface of the clypeus. Posterior to this is a median *anterior pharyngeal retractor muscle* (ant. phar. ret. mus.). This muscle has its origin on the frontal sclerite of the head capsule. A pair of *longitudinal pharyngeal muscles* (long. phar. mus.) connect the *anterior pharynx* (ant. phar.) with the more posterior part (post. phar.). At the posterior region of the pharynx and between the post-pharyngeal glands (post. phar. gls.) there is a *transverse pharyngeal muscle* (trans. phar. mus.). The remaining two muscles are paired. The *oblique pharyngeal muscles* (ob. phar. mus.) are inserted at the points where the glands open into the pharynx. These muscles have their origin on the clypeo-frontal apodemes near the bases of the antennae. *Posterior pharyngeal retractor muscles* (post. phar. ret. mus.) connect the pharynx with the vertex of the head.

FIG. 4. — Longitudinal section through part of the post-pharyngeal gland; Ehrlich's haematoxylin and eosin;
int., intima; *lum.*, gland lumen; *n*, cell nucleus; *cyt.*, cytoplasm.

III. — Anatomy and histology of the post-pharyngeal glands.

The paired *post-pharyngeal glands* (post. phar. gls.) of *Acromyrmex octospinosus* (fig. 1 and 2) consist of a proximal sac-like portion from which several digitations extend. The gland opens into both sides of the pharynx, just in front of the olfactory lobes of the brain. The openings of the glands into the pharynx are protected by rings of sclerotised cuticle which form part of the supporting cuticle of the post-pharyngeal region.

Histologically, the post-pharyngeal gland is very simple. In both parts of the gland the wall consists of a single layer of cuboidal epithelial cells supported by an extension of the foregut *intima* (int.) about 1 µm thick. In surface view (fig. 3) the cells (epi. c.) appear very regular and hexagonal, with spherical *nuclei* (n) about 4 µm in diameter, small in relation to the volume of *cytoplasm* (cyt.). The lumen of the gland contains a yellow oil. No further structures could be resolved with the light microscope.

IV. — Preliminary chemical analysis of the oil.

No difference between the composition of the lipids of the post-pharyngeal gland oil and those of a hexane-diethyl ether extract of whole dissected glands was detected.

Table I summarises the major components of the post-pharyngeal gland oil. There were also traces of diglycerides, sterol ester and an unidentified component which was only slightly less polar than the triglyceride fraction on TLC.

The identities of the major components of the gland were confirmed by TLC and mass spectrometric comparison with authentic standards. The chemical constituents of the post-pharyngeal gland are being analysed in more detail and will be described more fully in a later publication.

TABLE I. — APPROXIMATE CHEMICAL COMPOSITION OF THE MAIN COMPONENTS OF THE OIL IN THE POST-PHARYNGEAL GLANDS OF *Acromyrmex octospinosus*.

LIPID CLASS	CONCENTRATION	COMPONENTS
Triglycerides.	> 85 %	10 triglycerides constitute the major part of the triglyceride content of the gland. The main compound appears to bedioleoyl palmitin.
Free fatty acids.	< 5 %	Mainly palmitic acid.
Free sterols.	$\ll 5$ %	Mainly ergosterol.

DISCUSSION

The mode of action of the post-pharyngeal glands of ants has never been thoroughly investigated. For example, it is not certain whether the contents of the gland are secreted into the pharynx. The appearance of an oily secretion on the mouthparts has never been reported, although the proximity of the gland to the mouth suggests that this occurs.

The simplicity of the cellular structure of the gland and absence of compressor musculature rules out the possibility of a rapid discharge as occurs, for example, in arthropod defensive glands (EISNER, 1970). Changes in haemocoelic pressure may facilitate the secretion of the gland contents. Alternatively the contents may leak slowly into the alimentary tract. There are three pairs of muscles and a single median muscle in the post-pharyngeal region and possibly some or all of these are concerned with the regulation of gland discharge. The inferior muscles of the anterior pharyngeal region are almost certainly involved in ingestion.

The oil in the post-pharyngeal glands may not be wholly a secretory product of the glandular cells. BARBIER and DELAGE (1967) reported that the post-pharyngeal glands of *Messor* could drain liposoluble material from the ingested food. Earlier ECHOLS (1966) noticed that feeding the leaf-cutting ant *Atta texana* on dyed soya bean oil caused the post-pharyngeal glands to become heavily stained, but did not suggest any pos-

sible mechanisms. The same process occurs in *Acromyrmex octospinosus* and *Atta cephalotes* (PEREGRINE et *al.*, 1972). The oil in the gland of *Acromyrmex* is probably a mixture of lipids drained from the food with those produced by the secretory epithelium of the gland itself.

Acromyrmex feeds largely on the fungus cultured in the nest (WEBER, 1966). Ergosterol is abundant in the fungus cultured by *Acromyrmex* (R. T. Aplin, personal communication). This compound and possibly others, may be taken directly into the gland by the process outlined above.

The hypopharyngeal glands of the honeybee, which are considered to be homologous with the post-pharyngeal glands of ants (BORDAS, 1894), secrete amylase and invertase (KOSMIN and KOMAROW, 1932). A detailed study of the enzymology of the post-pharyngeal glands of ants might therefore prove rewarding.

The function of the post-pharyngeal glands of *Acromyrmex* and other ants has not been fully determined. Various theories have been proposed. By far the most popular is the suggestion that the oily secretion serves as food for the larvae (AYRE, 1963; 1967; BARBIER and DELAGE, 1967; MARKIN, 1970; NAARMANN, 1963; and PAULSEN, 1969). Only MARKIN and NAARMANN produced experimental evidence to support their claim. *Acromyrmex* larvae are normally fed on clusters of fungal hyphae cultured in the nest. Possibly this diet is supplemented by glandular secretions. However, FORBES and MCFARLANE (1961) claim that the secretion of the post-pharyngeal gland is not fed to the larvae, but is used by the individual ant for digestion. Thirdly, BRIAN and BLUM (1969) suggested that the oil is applied to the surface of larger larvae to regulate their growth. Possibly the post-pharyngeal glands serve different functions in different species of ants (AYRE, 1963).

The oil from the gland mostly consists of triglycerides. The high energy value of triglycerides would certainly support the suggestion that the oil has at least some nutritive role and the same may be true of the fatty acids present.

The occurrence of ergosterol in the post-pharyngeal glands of *Acromyrmex* is even more interesting because unlike mammals, insects cannot synthesise sterols from simpler compounds (CLAYTON, 1964) and a dietary supply of sterol seems essential for their growth and development (CLAYTON, 1964; GILBERT, 1967). The presence of ergosterol in the fungus could explain its presence in the glands. Ergosterol was not apparently found in the pharyngeal gland of the ant *Messor capitatus* (Latr.) which was shown (BARBIER and DELAGE, 1967) to contain phytosterols, mainly campesterol.

CLAYTON (1964) reviewed the utilisation of sterols in insects and commented on their probable functions. Sterols are probably essential components in subcellular membranes and this may be their most important function. They have also been shown to be important in reproduction in some insects e. g. *Musca domestica* (MONROE, 1959). Also the dietary sterols may be precursors of growth and moulting hormones (GILBERT, 1967). These functions would certainly justify the feeding of the gland contents to the larvae.

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